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FUNCTION OF SLEEP (C CIRELLI, SECTION EDITOR)

Sleep Homeostasis, Metabolism, and Adenosine

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Abstract Sleep is an integral and constitutive part of life, invariably observed in animals with even a simple nervous system. Importantly, sleep is an active and highly regulated state. Sleep propensity or sleep need and its best established biological marker, electroencephalographic (EEG) slow-wave (or delta) activity, is tightly associated to prior wakefulness and sleep and is homeostatically regulated. Sleep need may be considered an essential aspect of life, just like feeding, drinking, and procreation. Sleep, therefore, likely developed in a primordial state of evolution and should either aid or, at least, not interfere with other essential biological aspects of life such as metabolisms and reproduction. Consistent with this view, brain circuitries regulating sleep need, metabolism, and reward appear to involve the basal ganglia and are tightly linked. They may sense changes in the organism's major cellular energy store, adenosine-tri-phosphate (ATP), and its derivative adenosine, and act in concert with other important neuromodulatory systems including dopamine, glutamate, and hypocretin.

Keywords Adenosine receptors · Caffeine · ATP · Purinergic receptors · P2X · Dopamine · D_2 receptors · Metabotropic glutamate receptors · mGluR5 · Hypocretin (orexin) · Basal ganglia · Striatum · Receptor heteromers

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Introduction

Twenty years ago, in 1995, Benington and Heller [1] proposed that the function of sleep is to replenish brain energy stores and that extracellular adenosine levels provide the brain signal for measuring sleep need. This seminal and scholarly theory provided and inspired a number of testable hypotheses, some of which are still investigated today. The currently available results suggest that the roles for sleep in regulating energy metabolism are probably more complex than originally proposed [2, 3]. Nevertheless, it is now well established that adenosine and adenosine receptors play important roles in regulating wakefulness and sleep (see [4, 5] for recent reviews). This evidence, as well as some ideas on the intricate possible links among sleep homeostasis, metabolism, and adenosine, will be discussed in this overview.

Wakefulness and Sleep are Regulated by Circadian and Homeostatic Influences

The cyclic alternation between wakefulness and sleep forms a fundamental biological rhythm during the 24-h light-dark cycle. These two distinct states result from the interplay between circadian and homeostatic oscillators, a concept originally described by the two-process model of sleep–wake regulation [6]. The circadian process reflects an endogenous, 24-h variation in the propensity for wakefulness and sleep [6] and is controlled by the suprachiasmatic nuclei (SCN) of the anterior hypothalamus. The SCN is considered the circadian master clock in mammals. In humans, the SCN may promote wakefulness in the early evening, to prevent people from falling asleep during the time when homeostatic sleep pressure reaches its highest level after a waking day. The homeostatic process represents an hourglass mechanism, which gradually builds up with increasing time awake, and roughly exponentially declines during sleep. Thus, in healthy humans, circadian and homeostatic systems work in opposition to ensure

consolidated periods of wakefulness and sleep [7]. The interaction of the two processes also allows for consolidated sleep during the biological night. The SCN may promote a circadian increase in sleep tendency, which counteracts the decrease in homeostatic sleep propensity as the individual accumulates sleep [8, 9].

Multiple adenosine receptor subtypes appear to contribute to the control of clock functions and circadian timing. This evidence was recently summarized elsewhere [10]. Here, we will focus on the potential roles for adenosine and adenosine receptors in regulating the homeostatic facet of sleep and metabolism.

Adenosine and Adenosine Receptors

Adenosine Formation, Metabolism, and Clearance

Adenosine is usually regarded as a neuromodulator rather than a neurotransmitter because of the way it operates and is released in the nervous system. The formation of adenosine in the brain changes in an activity-dependent manner and is linked to the intracellular depletion of the energy-rich molecule adenosine-tri-phosphate (ATP) [11, 12]. Triggered by energy consumption, ATP is dephosphorylated to adenosine-di-phosphate, adenosine-mono-phosphate (AMP), and adenosine. Extracellular adenosine binds to one of four specific G-protein-coupled adenosine receptors. Caffeine, the world's most readily consumed psychostimulant compound, promotes wakefulness and inhibits sleep by antagonizing distinct adenosine receptors [13].

Changes in activity-dependent levels of extracellular adenosine depend on several factors [14]. When intracellular adenosine concentrations are high, adenosine can be released directly from neurons via equilibrative nucleoside transporters (ENTs) [15]. The main metabolic pathways of adenosine are the phosphorylation to AMP by adenosine kinase (ADK) and the irreversible degradation to inosine by adenosine deaminase (ADA). Adenosine can also be produced directly in the extracellular space through hydrolysis and dephosphorylation of ATP by ecto-nucleotidases [12, 16]. The ATP is released from γ-amino-butyric acid (GABA)ergic, cholinergic, monoaminergic, and glutamatergic neurons as a consequence of cellular activity [17, 18]. It may be noteworthy that, in rodents, the final step to release adenosine by dephosphorylation of AMP, occurs primarily in the striatum and olfactory bulb [19]. Especially the striatum, a main structure of the basal ganglia (BG), has recently been proposed to play important roles in sleep–wake regulation [20, 21]. Furthermore, ATP and adenosine can be released by a recently established process called gliotransmission. Molecular genetic manipulations in mice revealed that glial cells provide a significant source of extracellular adenosine in the brain [22, 23•], which is crucial for sleep and distinct sleep-dependent brain functions [24–26].

The neuromodulatory actions of adenosine are mainly terminated by neuronal and astrocytic (re)-uptake by the so-called non-concentrative nucleoside transporters [27]. On the other hand, ADA, which is mainly expressed on the extracellular membrane and on cell surface-bound adenosine receptors, is believed to importantly modulate receptor affinity for adenosine [28]. This enzyme may be of particular importance for adenosine clearance when elevated levels of the nucleoside have to be cleared, for example after prolonged wakefulness.

Adenosine Receptors

Four different subtypes of G-protein-coupled adenosine receptors mediate the cellular effects of adenosine: A_1, A_{2A}, A_{2B}, and A_3 receptors. Activation of A_1 and A_3 receptors inhibits adenylate cyclase (AC) via G_i-proteins, whereas A_{2A} and A_{2B} receptors are coupled to G_s proteins that increase AC activity [29]. The divergent second messenger pathways of A_1 and A_3 compared to $A_{2A/B}$ receptors, as well as their different expression patterns and affinities for endogenous adenosine, are important topics of current adenosine research (see [30] for an extensive recent review). The characteristics of the currently known adenosine receptor subtypes will be briefly summarized in the following paragraphs.

The A_1 receptors are expressed throughout the body and located widely on excitatory neurons in the brain where they are found on pre- and postsynaptic membranes. *In vivo* positron emission tomography (PET) with selective radioligands revealed highest A_1 receptor occupancy in striatum and thalamus, as well as in temporo-parietal and occipital cortices [31]. The affinity of A_1 receptors for adenosine is high (nanomolar range), and their activation can promote delta activity in nonrapid eye movement (NREM) sleep [32], inhibit lipolysis [33], reduce heart rate [34], and decrease renal blood flow [35]. A recent PET brain imaging study in humans suggested that four to five cups of coffee (corresponding to ~450 mg caffeine) in a 70-kg volunteer can displace endogenous adenosine from 50 % of cerebral A_1 receptors [36•].

The A_{2A} receptors have a similarly high affinity for adenosine, yet are expressed in high abundance primarily on post-synaptic membranes in the dorsal striatum [37]. A recent study mapped the projections of A_{2A}-receptor-expressing neurons of the nucleus accumbens (NAc) within the dorsal striatum [38•]. It revealed specific projections to ventral pallidum (VP), lateral hypothalamus, lateral preoptic area, raphe nucleus, ventral tegmental area (VTA), and other parts of the brainstem. Local activation of A_{2A} receptors in the NAc is suggested to promote sleep [39•] and has also been linked to reward, feeding, and other goal-oriented behaviors [40]. Importantly, caffeine not only blocks A_1

receptors but also competitively antagonizes A_{2A} receptors in a nonselective manner [13]. Studies in genetically modified mice suggests that A_{2A}, but not A_1, receptors mediate the wake-promoting effect of the stimulant [41]. In the periphery, A_{2A} receptors are found in the spleen and leukocytes, and to a lesser extent in heart, lung, and blood vessels. Their activation results in vasodilation and hypotension [42] and has also been associated with immunosuppression and inflammation [43, 44].

The A_{2B} receptors are widely expressed in brain and periphery, yet their abundance is low. Furthermore, the A_{2B} receptor has low affinity for adenosine (micromolar range). Endogenous activation of these receptors may only be observed in pathological conditions where adenosine levels are elevated, such as during hypoxia [45, 46] or ischemia [47, 48]. Nevertheless, the A_{2B} receptor may play a role for metabolic effects and modulate glucose homeostasis, blood lipid levels, and atherosclerosis [49, 50].

Knowledge about the A_3 receptor is scarce, and its role varies among different species. Generally, A_3 receptors are only expressed at low levels, show low affinity for adenosine [14] and have been suggested to play a more prominent role during development than later in life [51].

Sleep Homeostasis and Adenosine

Electrical brain activity as measured with the EEG provides distinct neurophysiological fingerprints of wakefulness, NREM sleep, and REM sleep. In deep slow wave sleep, high-amplitude slow waves (or delta waves) with a frequency of 0.5-2 Hz are most prevalent. The duration of slow wave sleep and the preponderance of EEG delta oscillations in NREM sleep (and also to some extent in REM sleep) reliably reflect the duration of prior wakefulness and provide the best established biomarker of sleep need and sleep homeostasis. Many studies in animals and humans demonstrated that EEG power density in the delta range, particularly in NREM sleep, is enhanced after prolonged wakefulness. The reversed pattern can be observed after a daytime nap or during the decline of "sleep intensity" in the course of a sleep episode [52]. Converging evidence accumulated over the last two decades indicates that adenosine and adenosine receptors contribute to the regulation of sleep intensity and, possibly, to sleep need and sleep homeostasis.

Does Adenosine Regulate Sleep Intensity and Sleep Homeostasis?

In animal models, extracellular adenosine levels in the brain, are often higher during the active phase (dominated by wakefulness) than during the rest phase (dominated by sleep) [53–55]. Adenosine fulfills major criteria of an endogenous sleep regulatory substance [56]. Briefly, intracerebroventricular infusion of adenosine promotes sleep in rats [57] and adenosine levels in hippocampus, cortex, and basal forebrain (BF) are increased after sleep deprivation and normalized after recovery sleep [58, 59]. In slices prepared from the rodent BF, these sleep–wake dependent changes were recently confirmed in vitro [60]. Furthermore, excitatory glutamatergic inputs from the hypothalamus, amygdala, and hippocampus to GABAergic and cholinergic neurons of the BF contribute to local adenosine production [60, 61]. These findings may further support the idea that the BF acts as an adenosine sensor to determine sleepiness or sleep need following wakefulness. Nevertheless, lesion and pharmacological studies revealed that accumulation of adenosine in the BF is not necessary for sleep induction, nor are BF cholinergic neurons essential for sleep drive [62]. These data challenge a causal role for adenosine in the BF as *the* regulator of sleep homeostasis. Furthermore, ATP and adenosine in the extracellular space are rapidly metabolized and removed and are, therefore, unlikely to be involved in long-term sleep–wake regulation [18]. The available evidence rather suggests that extracellular adenosine provides a global feedback signal on neuronal networks, including subcortical and cortical structures [23•, 63] and contributes to wake–sleep transitions and the regulation of important functional aspects of sleep such as sleep intensity.

Consistent with this view, genetic studies in mice and humans suggest that the adenosine metabolizing pathway contributes to the generation of EEG delta oscillations reflecting sleep intensity (Table 1). Transgenic mice with increased ADK activity (*Adk-tg*) show reduced EEG delta power and appear to have a reduced capacity to intensify sleep after sleep deprivation [64]. In addition, a functional polymorphism in the gene encoding ADA in humans, which causes a reduced breakdown of adenosine to inosine [77], has been consistently associated with prolonged slow wave sleep and enhanced EEG delta activity in NREM sleep [66, 67•, 68]. This observation first made in stringently controlled laboratory experiments was independently confirmed by a large, community-based epidemiological sample [69•]. By contrast, the homeostatic response to sleep deprivation was not reliably altered in *Adk-tg* mice and unaffected by *ADA* genotype in humans [64, 67•].

In conclusion, the current findings indicate that the adenosinergic tone at the synapse regulates sleep intensity. Nevertheless, it needs to be kept in mind that, in the available studies, information regarding adenosine levels in the brain is generally lacking [78]. The observed phenotypes could, therefore, reflect other changes in the adenosine metabolizing pathways and may not necessarily be due to altered adenosine levels.

Table 1 Genes of the adenosine signaling pathway investigated for their involvement in the regulation of delta activity in NREM sleep (delta power phenotype) and sleep homeostasis (homeostatic phenotype)

Gene	Species	Genetic evidence/model	Delta power phenotype	Homeostatic phenotype	Comment	Reference(s)
Adk	Mouse	*Adk-tg*	Yes	Yes		[64]
Ada	Mouse	Chromosome 2 (QTL analyses)		Yes	Rebound in delta power after sleep deprivation	[65]
ADA	Human	SNP rs73598374	Yes		Controlled baseline sleep recordings	[66, 67•, 68]
	Human	SNP rs73598374	Yes		Epidemiological study	[69•]
	Human	SNP rs73598374		No	Controlled sleep deprivation studies	[67•, 68]
AdoR	Drosophila	$dAdoR^{-/-}$		No	Pharmacogenetics of caffeine	[70]
A_1R	Mouse	$A_1R^{-/-}$	No	No	Constitutional KO	[71]
	Mouse	$A_1R^{-/-}$	Yes	Yes	Local conditional KO	[72]
	Mouse	*dnSNARE*	Yes	Yes	Disrupted adenosine release from astrocytes	[24, 26]
$A_{2A}R$	Mouse	$A_{2A}R^{-/-}$	No	Yes		[73]
ADORA2A	Human	SNP rs5751876	No		Baseline sleep recording	[74]
	Human	8 SNPs (Haplotype HT4)		Yes	Pharmacogenetics of caffeine	[75••, 76]

The observations of altered sleep and homeostatic phenotypes are based on the conclusions of the authors of the cited publications

Adk adenosine kinase, *Ada ADA*, adenosine deaminase, *SNP* single nucleotide polymorphism, *AdoR* adenosine receptor, A_1R adenosine A_1 receptor, *KO* knock-out, *dnSNARE* dominant-negative expression of the cytoplasmic domain of synaptobrevin II, $A_{2A}R$, *ADORA2A* adenosine A_{2A} receptor, *CNS* central nervous system

Do Adenosine Receptors Regulate Sleep Intensity and Sleep Homeostasis?

One of the most common indications that adenosine signaling contributes to the control of sleep intensity comes from the powerful wake-promoting effects of caffeine, which also attenuates waking and sleep EEG markers of sleep homeostasis [75••, 79]. Nevertheless, while the effects of caffeine on sleep may be independent from adenosine receptors in *Drosophila* [70], the specific contributions of A_1 and A_{2A} receptors also remain controversial in mammals [21].

Adenosine A_1 Receptors

It has long been thought that the adenosine A_1 receptors, which are widely distributed in the central nervous system (CNS), are responsible for the sleep-promoting effects of adenosine [1]. Prolonged wakefulness appears to be associated with upregulated A_1 receptor binding in subcortical and cortical brain structures in rats and humans [31, 80, 81]. Nevertheless, a careful study performed in A_1 receptor knockout mice revealed that homeostatic sleep–wake regulation is unaltered in animals that constitutively lack A_1 receptors [71]. More recently, a CNS-specific conditional knockout of this receptor was created. This genetic model showed reduced delta power in NREM sleep both in baseline and recovery sleep opportunities following sleep deprivation [72]. The authors concluded that elevated sleep need is, at least in part, signaled through A_1 receptors. Nevertheless, as discussed in a recent overview [78], the sleep restriction protocol employed may have allowed the mice to recover from sleep loss and to reduce delta power between the sleep deprivation periods. Furthermore, a general reduction in delta power in wakefulness, NREM sleep, and REM sleep was reported, suggesting that this gene impacts general electrical activity of the brain rather than having a specific effect on sleep homeostasis.

A recent study investigated the presynaptic signaling cascade of A_1 receptors and the effects of adenosine on $Ca_V2.1$ voltage-dependent calcium channels in a transgenic mouse line [82•]. These channels are predominantly localized on presynaptic nerve terminals where they play a key role in mediating neurotransmitter release. They are specifically susceptible to G_i-protein-coupled neurotransmitter inhibition, for instance by activation of A_1 receptors. The findings of Deboer and co-workers suggest that inhibition of $Ca_V2.1$ channels by adenosine contributes to increased sleep propensity, sleep initiation, and the sensitivity of caffeine. The data highlight the possibility that the wake-promoting effects of adenosine not only depend on altered postsynaptic signaling but also on ionotropic presynaptic signal transduction [82•].

Adenosine A_{2A} Receptors

Accumulating evidence indicates that A_{2A} receptors also contribute to the effects of adenosine on sleep. Mice lacking functional A_{2A} receptors show an absent sleep rebound after sleep deprivation [83], and A_{2A} receptors in rats appear to be downregulated by sleep loss [81]. Furthermore, local administration of a selective A_{2A} receptor agonist in the vicinity of

the ventrolateral preoptic area and NAc of the hypothalamus [84], as well as in the pontine reticular formation [85], promotes NREM sleep. Recently, studies in conditional knockout mice revealed that A_{2A} receptors in the shell region of the striatal NAc may play an essential role in the wakefulness-promoting effects of caffeine [39•].

Importantly, findings in humans also support a role for adenosine A_{2A} receptors in sleep–wake regulation. More specifically, genetic variation of the A_{2A} receptor (*ADORA2A*) gene was shown to modulate the sleep-deprivation-induced increase in EEG delta activity in NREM sleep, as well as subjective and objective effects of caffeine on the sleep EEG [74, 75••]. A role for the common 1976T>C polymorphism (rs5751876) of *ADORA2A* for caffeine-related sleep disturbances was recently confirmed in a large genome-wide-association study [86••].

In conclusion, although contradictory findings exist, the current evidence indicates that both A_1 and A_{2A} receptors contribute to the regulation of sleep intensity and sleep homeostasis (Table 1). The two adenosine receptor families may act in region-specific manner, and their effects may not be independent. Indeed, an interesting recent PET brain imaging study in humans shed new light on the possible functional consequences of genetic variants of *ADORA2A*. The study shows that the rs5751876 T-allele, which has been associated with caffeine insensitivity [74] and a stronger increase in EEG delta activity after sleep deprivation [74, 75••], corresponds to higher A_1 receptor availability in the brain [87••]. These data suggest that the sleep-related effects of *ADOARA2A* polymorphism rs5751876 may not reflect A_{2A} receptor function alone, but could also be linked to differential A_1 receptor expression.

Purine Type-2 (P2X) Receptors

As mentioned above, extracellular adenosine is rapidly metabolized and removed. It has, therefore, been questioned whether adenosine alone can be responsible for long-term sleep–wake regulation [18]. Adenosine is released in response to neuronal activity and may modulate sleep–wake regulatory processes indirectly via ATP. The activity-dependent transient changes in extracellular ATP are slower and can be detected by membrane purine type-2 (P2X) receptors. For example, independently of adenosine, $P2X_7$ receptors cause the release of other, activity-dependent sleep regulatory substances, such as brain-derived neurotrophic factor, which also modulate sleep intensity and sleep homeostasis [88].

Adenosine Receptor Heteromers and Receptor–Receptor Interactions

An important and currently intensively investigated aspect of adenosine signaling is the ability of adenosine receptors to form heteromers and receptor–receptor interactions. In striatal astrocytes, A_1–A_{2A} receptor heteromers are observed, and their activation may depend on the concentration of adenosine. At low adenosine concentrations, the activation of G_i-protein coupled A_1 receptors in the A_1–A_{2A} oligomer will predominate, whereas at high adenosine concentrations, A_1 receptors are inhibited, leading to the preferential activation of G_s-protein coupled A_{2A} receptors [89, 90]. Furthermore, adenosine A_1 and A_{2A} receptors form functional heteromers with dopamine D_1 and D_2 receptors. In both cases, binding of adenosine results in reduced dopaminergic signaling. The A_1–D_1 receptor heteromers are preferentially expressed in the so-called *direct pathway* on striato-nigral neurons in the BG [91, 92]. By contrast, A_{2A}–D_2 heteromers are mainly expressed on striato-pallidal neurons originating in VTA and NAc, which are part of the so-called *indirect pathway* [21, 92]. The NAc has been suggested to play a crucial role in sleep–wake regulation, by integrating signals from cortex, thalamus, amygdala, and midbrain dopaminergic neurons [21, 93, 94]. The co-localization of adenosine and dopamine receptors may allow the NAc to integrate adenosinergic and dopaminergic interactions to promote sleep. The NAc is ideally located and inhibits via GABAergic interneurons four pathways of the brain's arousal and activation system (Fig. 1). These pathways include the following: (1) VP, thalamus, and medial prefrontal cortex (mPFC), a pathway important for cognition and emotional processes, which are sensitive to sleep need; (2) lateral hypothalamus (LHA), tuberomammillary nucleus (TMN), and locus coeruleus (LC), an essential pathway for maintaining wakefulness; (3) parabrachial nucleus (PB) and BF, a pathway involved in maintaining wakefulness and cognitive performance; and (4) dopaminergic neurons of the VTA, which feeds back to the NAc, primarily via dopamine D_2 receptors [21, 94]. According to this model proposed by Lazarus and colleagues, stimulation of the VTA promotes wakefulness by activating inhibitory dopamine D_2 receptors of the NAc, which results in a disinhibition of the four activating pathways. By contrast, activation of excitatory A_{2A} receptors of the NAc actively promotes sleep by inhibiting the four activating pathways.

In accordance with these hypotheses, dopamine levels in mPFC and NAc of freely moving rats were reported to be elevated in wakefulness and REM sleep, yet to be reduced in NREM sleep [95]. Elimination of dopamine D_2 receptors in mice reduces wakefulness and enhances sleep [96], whereas either activation or blockage of dopamine D_2-type receptors in the NAc enhances wakefulness or sleep, respectively [97]. Finally, genetic removal of the murine dopamine transporter (DAT), a presynaptic protein responsible for reuptake of dopamine primarily in the BG, is associated with enhanced sleep and hypersensitivity to wake promotion by caffeine [98].

These preclinical observations were recently corroborated in humans. More specifically, brain imaging studies revealed that dopamine D_2 receptors are downregulated in NAc and

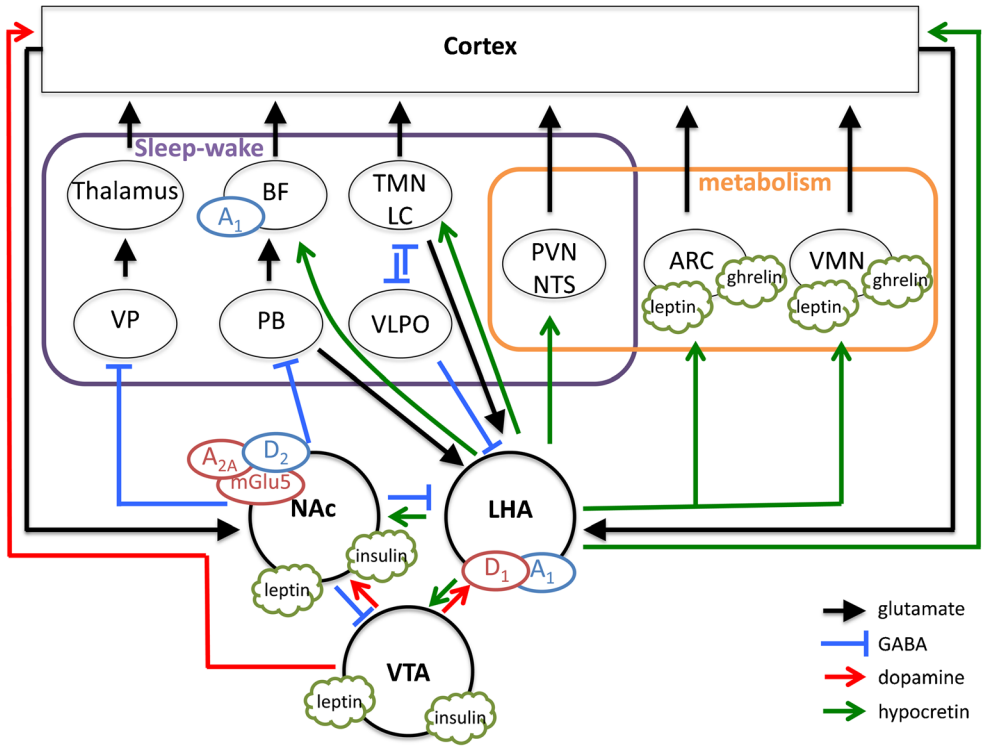

Fig. 1 Schematic representation of adenosine dependent basal ganglia projections involved in sleep wake regulation (*dark purple*) and metabolism (*orange*), and their modulation by adenosinergic, dopaminergic, and glutamatergic receptor heteromers. The model proposes that adenosine by activating excitatory (*red circles*) A_{2A} receptors (or glutamate by activating metabotropic glutamate type 5 [mGlu5] receptors) in the nucleus accumbens (NAc), can inhibit brain pathways, essential for arousal and feeding behavior, via GABAergic neurotransmission. Oppositely, activation of inhibitory (*blue circles*) dopamine D_2 receptors by dopaminergic projections from the ventral tegmental area (VTA) reduces NAc activity and thereby promotes wake and feeding behavior. Secondly, activation of inhibitory adenosine A_1 receptors in the lateral hypothalamus (*LHA*) inhibits LHA activity and thereby promotes sleep and fasting. Activation of LHA excitatory dopamine D_1 receptors, on the other hand, promotes LHA activity and thereby promotes arousal and metabolic pathways. Finally, insulin and leptin, which signals the availability of food, are able to promote feeding and inhibit sleep by directly binding to the NAc and VTA. Taken together, the dopaminergic and adenosinergic modulations of the NAc and LHA are ideally positioned within the basal ganglia to modulate sleep–wake regulation and metabolism. *VP* ventral pallidum, *VLPO* ventrolateral preoptic nucleus, *TMN* hypothalamic tuberomammillary nucleus, *LC* locus coeruleus, *NTS* nucleus tractus solitarius, *BF* basal forebrain, *PB* Parabrachial nucleus, *PVN* paraventricular nucleus, *ARC* arcuate nucleus, *VMN* ventro-medial nuclei, *NAc* nucleus accumbens, *LHA* lateral hypothalamus (hypocretin), *VTA* ventral tegmental area (dopamine)

ventral striatum following sleep deprivation and that the decrease correlates with reduced cognitive performance [99, 100]. Furthermore, the functional polymorphism rs28363170 of the gene encoding DAT (*SLC6A3* or *DAT1*) modulates neurophysiological markers of sleep homeostasis and the effects of caffeine on these markers in NREM sleep [101•]. The fact that genetically determined alterations in striatal dopaminergic neurotransmission modulates the actions of caffeine to promote wakefulness in rodents and humans highlights the important interplay between adenosine and dopamine in sleep–wake regulation. While other dopaminergic influences may also be important [102, 103], these findings may be consistent with an integrative role played by the NAc, and a reciprocal interplay between A_{2A}- and D_2-receptor mediated signals in regulating wakefulness and sleep.

Finally, A_{2A} receptors have also been reported to form functional heteromers with subtype-5 metabotropic glutamate (mGlu5) receptors. The stimulation of mGlu5 receptors in A_{2A}–mGlu5–D_2 receptor heteromers causes reduced affinity of D_2 receptors for dopamine, and co-stimulation of both mGlu5 and A_{2A} receptors reduces their affinity even further [104]. Concerted activation of both mGlu5 and A_{2A} receptors in these receptor heteromers may, therefore, efficiently reduce arousal and promote sleep. In accordance with this hypothesis, the functional availability of mGlu5 receptors in a striatal region including the NAc is enhanced compared to baseline after the experimental deprivation of one night of sleep in humans [105]. Intriguingly, the same procedure was found to reduce dopamine $D_{2/3}$ receptor availability in the ventral striatum by almost the identical magnitude (~5 %) [100]. Taken together, the convergent findings may indirectly support the notion that A_{2A}–mGlu5–D_2 receptor heteromers modulate the quality of wakefulness and sleep via the NAc.

Metabolism and Adenosine

Adenosine in its basic form is an energy and ATP derivate, released from all major organs including liver, fat tissues, pancreas, and muscles. As such, it is not surprising that adenosine and adenosine receptors have been associated with the regulation of various metabolic effects in the periphery. These effects include insulin secretion from the pancreas, glucose release, glucose clearance, glycogenolysis, glycogenesis, insulin-mediated inhibition of lipolysis, leptin release from adipocytes, inhibition of ghrelin release, as well as enhancement of cholesterol synthesis in the liver (see [106] for recent in-depth review).

One hypothesis concerning the functions of sleep is that sleep is needed to conserve energy and enhance energy stores. Indeed, the regulation of metabolism by the brain has been repeatedly linked to sleep–wake regulation. This mutual relationship can be illustrated by the findings that energy demands are increased during prolonged wakefulness, whereas energy expenditure is reduced during sleep [107]. By reflecting ATP breakdown, adenosine may be an indicator of activity-dependent neuronal energy use. In accordance with a role for sleep in restoring brain energy, recent data in rats indicate that brain regions with predominantly wake-active neuronal activity such as the LHA undergo a surge in ATP during the initial hours of spontaneous sleep [108]. Interestingly, this spontaneous sleep-related ATP surge correlates positively with homeostatic sleep pressure as reflected in EEG delta activity in NREM sleep.

The neuropeptide hypocretin, which is released from the LHA, is one of the major molecules involved in feeding, temperature regulation, and metabolism. The LHA widely projects to cortex, paraventricular nucleus (PVN), nucleus tractus solitarius (NTS), NAc, VTA, as well as other parts of the ascending arousal system (Fig. 1) [21, 94, 109, 110]. Indeed, hypocretin is also a key player in regulating wakefulness and sleep. Activation of hypocretin neurons via hypocretin receptors maintains wakefulness and results in enhanced feeding, whereas loss of hypocretin neurons causes narcolepsy (see [110, 111] for recent reviews). Interestingly, adenosine can modulate the activity of hypocretin neurons of the LHA. Local injection of the selective A_1 receptor antagonist 1,3-diethyl-8-phenylxanthine (DPX) enhances activity in hypocretinergic neurons and promotes waking [112]. By contrast, activation of A_1 receptors in the LHA by adenosine promotes sleep [113, 114]. These findings illustrate that adenosine may contribute to sleep–wake regulation, as well as metabolism and feeding by modulating the activity of hypocretin neurons in vivo.

Other important areas for the regulation of metabolism and appetite are the ventro-medial (VMN) and arcuate (ARC) nuclei of the hypothalamus, which integrate a variety of peripheral and central signals including inputs from hypocretin neurons of the LHA [109]. The ARC modulates appetite mainly via the two hormones leptin and ghrelin. Leptin is released from adipocytes after food intake and mediates the perception of satiety. Ghrelin, on the other hand, is produced in cells of the stomach and intestines. Ghrelin levels rise during fasting and fall rapidly after food intake [115]. Apart from the ARC, neurons within the PVN and the NTS are important for satiety, taste, and autonomic functions controlling energy balance [109]. Given that adenosine modulates the activity of hypocretin neurons of the LHA, adenosine may play an indirect role in regulating satiety and appetite through these nuclei and pathways (Fig. 1), although direct evidence for this remains scarce.

A key element of metabolism is our ability to balance food intake with actual energy output. This motivational aspect of feeding is mainly associated with dopaminergic neurotransmission from VTA to NAc, which modulate hedonic and non-homeostatic food intake [109]. Food and water, or even just cues of their availability, promote dopaminergic firing of the VTA [116]. Moreover, insulin and leptin, which reflect the abundance of circulating carbohydrates and fat, can directly inhibit VTA activity and NAc signaling. By contrast, ghrelin, signaling hunger, can activate the VTA and promote dopaminergic NAc signaling. Evidence for a role of adenosine in modulating dopamine-dependent feeding behavior, comes from caffeine studies in rats. When administered orally or intraperitoneally, caffeine reduced food intake [117, 118]. Nevertheless, because adenosine receptors are widely expressed in the periphery and also modulate peripheral metabolism [106], these studies should be interpreted with caution. On the other hand, as explained above, the actions of dopamine in VTA and BG depend largely on adenosine, and especially on adenosine A_{2A} receptors. Thus, adenosine may be excellently positioned to not only regulate sleep intensity and sleep homeostasis but also to modulate the balance between energy intake and starvation (Fig. 1). The exact roles of the distinct adenosine receptors in these processes are complex and are subject of intense ongoing research.

Conclusion

The possible roles for adenosine and adenosine receptors in regulating wakefulness and sleep were an area of active research during the past two decades. Although it is now widely accepted that endogenous adenosine acts as a sleep regulatory substance, the actions of adenosine in cortical and subcortical areas of the brain are complex and region specific. Accumulating evidence suggests that both A_1 and A_{2A} receptors modulate sleep intensity and sleep homeostasis. Much of this evidence stems from experiments in vitro and in animal models and has also been corroborated in humans.

Nevertheless, causal roles for adenosine in long-term aspects of sleep–wake regulation, such as sleep homeostasis, remain controversial, especially because of its rapid synaptic removal and degradation. Thus, associated changes in adenosine signaling pathways are also under investigation. It is more and more appreciated that adenosine receptors form functional receptor heteromers with dopamine and metabotropic glutamate receptors. They may be ideally positioned, for example in NAc and LHA, not only to regulate wakefulness and sleep but also to regulate metabolism and food intake. Nevertheless, important open questions remain, highlighting the importance of future research to elucidate the exact roles for adenosine in sleep homeostasis and metabolism. This research has important implications for society and public health because the adenosine receptor antagonist, caffeine, is readily available and the most widely consumed stimulant in the world.

Compliance with Ethics Guidelines

Conflict of Interest Sebastian C. Holst and Hans-Peter Landolt declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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